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Analysis of the Binding Specificities of Oligomannoside-binding Proteins using Methylated Monosaccharides

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Abstract—The binding specificities of the closely related lectins from *Canavalia ensiformis* and *Dioclea grandiflora* were examined using specifically *O*-alkylated mono- and disaccharides. Both lectins accept any substitution at the monosaccharide C2 hydroxyl group. The binding energy of C2-alkylated ligands–concanavalin A complexes increases by 1 kcal mol^{-1} for the C2-*O*-ethyl ligand, while the binding energies of the corresponding complexes with the *Dioclea* lectin are identical. Both lectins accept methyl, but not ethyl, substitution of the C3 hydroxyl, in contrast to earlier reports. The results are interpreted in terms of existing models of the concanavalin A binding site. While the results are consistent with a model of the concanavalin A extended binding site that places the non-reducing terminus of all disaccharides in the monosaccharide binding site, they point to the dangers of interpreting the binding behavior of unnatural saccharide ligands on the basis of crystallographic data obtained with native ligands. Copyright © 1996 Elsevier Science Ltd

Introduction

Protein–carbohydrate interaction is now widely recognized as an important recognition mechanism in a variety of biological systems.[1–6] In addition to controlling many aspects of normal cell biology, carbohydrate-based recognition plays important roles in myriad human disease states. For example, in the earliest phases of infection by many viral, parasitic, mycoplasmal and bacterial pathogens, the pathogen identifies and adheres to its host through a protein–carbohydrate interaction.[7–9] Protein–carbohydrate interactions also play several roles in cancer biology, including control of cell growth and differentiation, and direction of the non-random metastatic distribution of malignancies.[10–12] The galectins, for example, appear to be involved in the regulatory pathways of both normal cell growth and differentiation, and in the rapid cell proliferation following malignant transformation. Tissue-specific endogenous tumor lectins direct some non-random metastatic processes, while homologous tumor lectins directed towards the unique glycoforms found on tumor cells may be involved in the 'clumping' phenomena vital to metastasis.

Because of the roles played by carbohydrate-based recognition in human disease, there exists an opportunity to develop new therapeutic strategies based on carbohydrate-based interference of biological recognition. In such an approach, small-molecule synthetic carbohydrates or carbohydrate mimics could be effective therapeutics for a variety of unrelated diseases. Such species would act not as cytotoxins, but by blocking intercellular communication.

The intellectual appeal of such a strategy notwithstanding, there remain fundamental barriers to the development of carbohydrate therapeutics. Key among these are issues of tight binding and specificity. Protein–carbohydrate complexes are only weakly bound, with dissociation constants in the millimolar to micromolar range. Clearly, such weak associations are incompatible with in vivo therapeutic use, and general paradigms for the development of agents with vastly improved binding affinities are required. A second consequence of weak binding is that lectins generally show broad substrate specificities, binding a range of related structures with similar affinities. As a result, there exist in vivo multiple lectins with overlapping binding specificities. Clearly, therapeutic agents designed to interrupt biological communication must possess sufficient specificity to interrupt only the interaction of interest. Fundamental research directed towards these problems forms the core of our research interests and those of several other groups.[13–17]

Our approach to the problems of tight binding and specificity has been to study the binding of a model system of lectins with overlapping binding specificities to a range of natural and synthetic carbohydrate ligands.[15] We have studied the interactions calorimetrically: such studies provide direct measures of binding constants, as well as changes in enthalpy and heat capacity that accompany binding. From the derived free energies of binding, entropies of binding are obtained by subtraction. Calorimetric evaluation of complexation thus provides a complete thermodynamic description of the binding event. More recently we and others have developed experimental protocols for separating enthalpies of binding into solute–solute and solvation-associated phenomena.[18–20] In conjunction with work in the protein folding community providing a method for separating the solvation-associated entropy from the overall entropy of binding,[21–23] these techniques allow an unprecedented ability to interpret

bulk thermodynamic parameters in terms of intermolecular interactions.

Of the members of our model system, the lectins from the leguminous plants *Canavalia ensiformis* (concanavalin A) and *Dioclea grandiflora* are perhaps of greatest interest. Concanavalin A is the best studied of all lectins, and was first crystallized by Sumner in 1919.[24] Since that time, hundreds of studies of the structure and properties of the lectin have been reported.[25,26] Although first recognized as a glucose/mannose specific lectin, Brewer and co-workers reported in 1983 that the trimannoside methyl 3,6-di-*O*-(α-mannopyranosyl)-α-mannopyranoside was bound by the protein nearly 2 orders of magnitude more tightly than mannose.[27] Today, it is generally accepted that the trimannoside is the minimum carbohydrate structure that completely fills the carbohydrate binding site. In 1989, Derwenda et al. reported a crystal structure of the concanavalin A–methyl α-mannopyranoside cocrystal.[28] More recently, Naismith and Field reported the structure of the concanavalin A–trimannoside complex.[29]

In 1983, Moreiera and coworkers reported a lectin from the Brazilian legume *Dioclea grandiflora* closely related to concanavalin A.[30] The two proteins differ by only 59 of 237 amino acids, with no gaps. Only one of the substitutions is within 6 Å of the concanavalin A monosaccharide binding site. In contrast to other legume lectins, the *Dioclea* lectin shares with concanavalin A a specificity for the trimannoside. The close relation of the two proteins makes them ideal as a model for studying issues of specificity. Differentiating between two so closely related proteins is a demanding test of our ability to design synthetic ligands specific for a single protein in the presence of other lectins with closely related binding specificities.

Recently we proposed a concanavalin A binding site description that provides a model for protein–carbohydrate interaction, without specifying any specific locus on the protein surface.[15] In this model, methyl α-mannopyranoside and all of the mannobiosides are bound with the non-reducing terminal mannose in a single high-affinity site, presumably the crystallographic monosaccharide binding site. The reducing terminus of the disaccharides appears to contribute little, if anything, to the overall interaction. One arm of the trimannoside is also bound in this same site, and the remaining non-reducing terminus completes the extended protein–carbohydrate interaction.

Here we report the continuation of our studies comparing the binding properties of the lectins from *Canavalia* and *Dioclea*. In this work, we explore the steric and hydrogen bonding requirements of both proteins using a series of *O*-alkyl mono- and disaccharides (Fig. 1). While our results do not allow unambiguous determination of the nature of the binding site, they are consistent with our model, and at the same time point to the dangers of extrapolating crystallographic data to new ligands by simple group replacement.

Results and Discussion

Synthesis of substrates

2-*O*-Alkyl mannosides were prepared by alkylation of intermediate 19 (Scheme 1). Conversion of methyl α-mannopyranoside to the 4,6-*O*-benzylidene followed by selective allylation of the C3 hydroxyl via the stannylene, according to the method of Nashed, furnished the required C2 unblocked species.[31] Normal alkylation

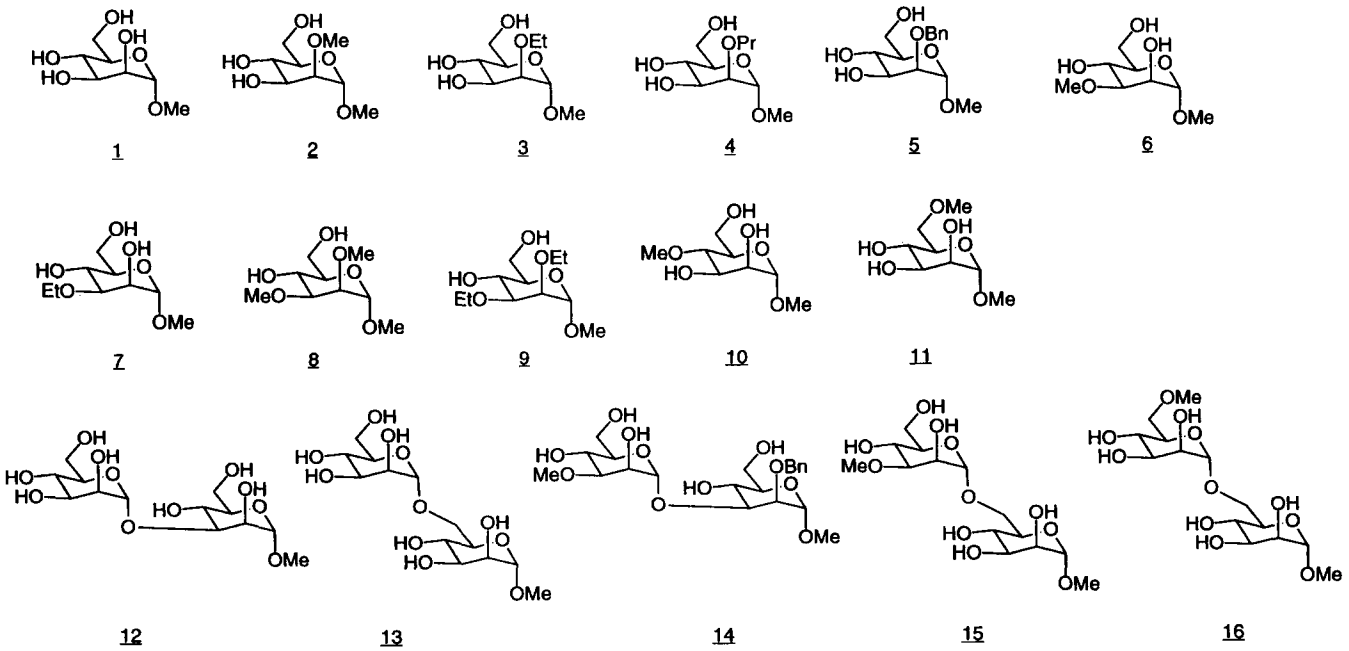


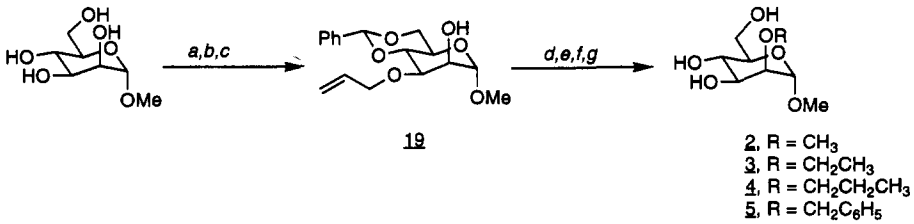
Figure 1. Ligands used in this study.

and deprotection gave methyl 2-*O*-methyl-, ethyl-, propyl-, and benzyl-α-mannopyranoside.

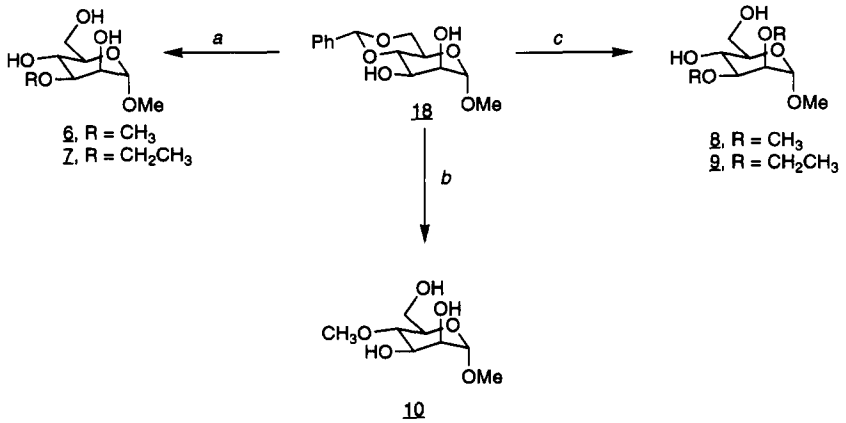
In similar fashion, methyl 4,6-*O*-benzylidene-α-mannopyranoside was selectively alkylated with both methyl and ethyl iodide, again via the stannylene. Deprotection gave methyl 3-*O*-methyl- and ethyl-α-mannopyranoside (Scheme 2). Benzylation of the C2 and C3 hydroxyls of methyl 4,6-*O*-benzylidene-α-mannopyranoside, removal of the benzylidene, selective silylation of the primary C6 hydroxyl moiety and methylation of the remaining C4 hydroxyl group provided the fully protected 4-*O*-methyl mannoside (Scheme 2). Finally, alkylation of the C2 and C3 hydroxyl groups of methyl 4,6-*O*-benzylidene-α-mannopyranoside with both methyl and ethyl iodide followed by removal of the benzylidene furnished methyl 2,3-di-*O*-methyl- and ethyl-α-mannopyranoside, respectively (Scheme 2).

Methyl 6-*O*-methyl-α-mannopyranoside was prepared by selective silylation of methyl α-mannopyranoside, perbenzylation of the C2, C3, and C4-hydroxyl moieties, desillyation and methylation of the C6-hydroxyl (Scheme 3). Debenzylation provided the required methylated monosaccharide.

The disaccharides **14**–**16** were prepared using thioglycoside coupling strategies. For the preparation of methyl 3-*O*-(3-*O*-methyl-α-mannopyranosyl)-α-mannopyranoside, we required thiomethyl glycoside **22**. Conversion of methyl α-1-thiomannopyranoside[32] to the 4,6-*O*-benzylidene, selective methylation of the C3 hydroxyl by methyl iodide treatment of the stannylene and acetylation of the remaining C2-hydroxyl yielded the required glycosyl donor (Scheme 4). The required glycosyl acceptor was prepared from methyl 2-*O*-benzyl-3-*O*-allyl-4,6-*O*-benzyllidene-α-mannopyranoside (**23**) by deallylation with Wilkinson's catalyst followed by oxymercuration. Coupling was effected under the aegis of *N*-iodosuccinamide (NIS) and triflic acid. Complete deprotection furnished the methylated disaccharide **14**.



Scheme 1.



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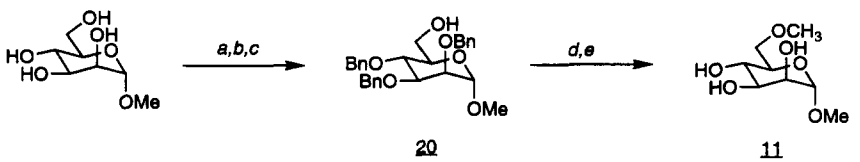

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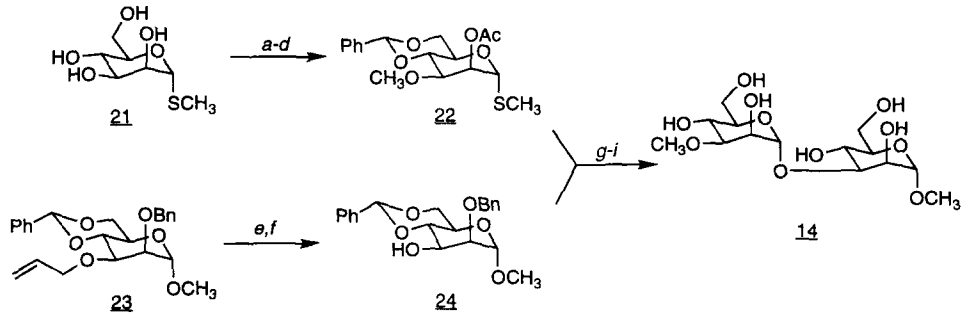


Scheme 4.

The same glycosyl donor was coupled to methyl 2,3,4-tri-*O*-benzyl-α-mannopyranoside (**20**, Scheme 5), again using NIS/triflic acid promotion. Complete deprotection provided the methylated disaccharide methyl 6-*O*-(3-*O*-methyl-α-mannopyranosyl)-α-mannopyranoside.

Preparation of methyl 6-*O*-(3-*O*-methyl-α-mannopyranosyl)-α-mannopyranoside required the glycosyl donor methyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-1-thio-α-mannopyranoside (**26**, Scheme 6). Conversion of methyl 1-thio-α-mannopyranoside to the 6-*O*-*t*-butyldiphenylsilyl derivative, benzylation of the C2-, C3-, and C4-hydroxyl moieties, desilylation and subsequent methylation of the C6-hydroxyl functionality provided the necessary glycosyl donor. Coupling of this donor to methyl 2,3,4-tri-*O*-benzyl-α-mannpyrannoside with NIS/triflic acid promotion, and complete deprotection gave the final methylated disaccharide **16**.

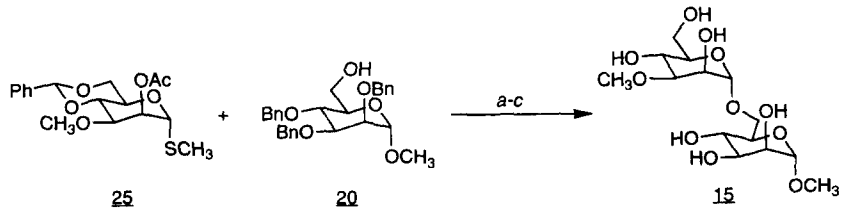
Protein purification

Lectins were isolated from *Canavalia ensiformis* and *Dioclea grandiflora* seed meal by affinity chromatography on Sephadex G75 as previously described.[15] Briefly, crushed seed was extracted with buffer, applied to a Sephadex column and after elution of contaminating proteins with buffer, the lectins were eluted with 100 mM glucose.

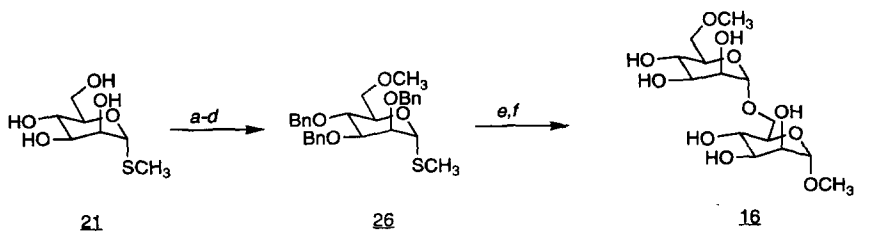
Titration microcalorimetry

Titration microcalorimetry was performed using the MicroCal Omega titration microcalorimeter. Details and performance of the instrument are reported elsewhere.[33] In each case, lectin at concentrations of 0.27–0.37 mM was loaded into the calorimeter cell. Carbohydrate at concentrations of 10–28 mM were delivered in 25–40 2.2 μL aliquots during 60–120 minutes. The enthalpy evolved on each injection was measured and digitally stored.

Details of data analysis using the Origin data analysis software for both the general case[33,34] and specifically for protein–carbohydrate binding have been reported elsewhere by us and others.[15] Briefly, the incremental heat dq evolved during a titration is related to the enthalpy of binding ΔH and cell volume V_0 by the expression:



Scheme 5.



Scheme 6.

$dq = d[complex]\ \Delta H\ V_o$.

The amount of complex formed during each injection is related to the known quantities $[lectin]_{total}$ and $[ligand]_{total}$ by the equilibrium expression:

$$K_{eq} = \frac{[complex]}{[lectin][ligand]}$$

and the mass balance equations

$[lectin]_{total} = [lectin] + [complex]$

$[ligand]_{total} = [ligand] + [complex]$.

The total heat content of the solution after the i^{th} injection Q_i, corrected for dilution, is given by the expression:

$$Q_i = \frac{[lectin]_{total}\ \Delta H\ V_o}{2}(1 + L_r + r - 1\sqrt{(1 + L_r + r)^2 - 4L_r}),$$

where $1/r = [lectin]_{total}\ K_{eq}$ and $L_r = [ligand]_{total}/[lectin]_{total}$. A non-linear least squares fit to the data to this expression that continuously varies K_{eq} and ΔH provides values of binding constants and enthalpies.

The binding constants, changes in free energy, enthalpy and entropy as well as ligand and lectin concentrations for binding of carbohydrates **1–16** to both concanavalin A and the lectin from *Dioclea* are shown in Tables 1 and 2.

The data can be interpreted in terms of the recently published structure of the concanavalin A extended binding site, as well as our own model of legume lectin–oligosaccharide interaction.

2-*O*-Alkyl monosaccharides

It has long been recognized that concanavalin A displays a relaxed specificity for substituents at C2 of the monosaccharide structure. Thus, concanavalin A binds both glucose and mannose, and binds the disaccharide methyl 2-*O*-(α-mannopyranosyl)-α-mannopyranoside roughly 100-fold more tightly than the monosaccharide methyl α-mannopyranoside. In contrast, the lectin from *Dioclea* shows only a 2-fold preference for the 1→2 mannobioside. Neither lectins, however, bind either *N*-acetylglucosamine or *N*-acetylmannosamine.

The only amino acid substitution in the *Dioclea* lectin relative to concanavalin A proximal to the monosaccharide binding site is the residue at 226, a threonine in concanavalin A and glycine in the *Dioclea* lectin. While the threonine hydroxyl does not appear to form a hydrogen bond with the mannopyranoside,[28] it does form something of a pocket in the space immediately adjacent to the C2 hydroxyl (Fig. 2). We reasoned, then, that extending alkyl substituents into the space adjacent to C2 of the carbohydrate ligand may be acceptable to both lectins. Our prediction was borne out by the behavior of the C2-*O*-methyl-, ethyl-, and propyl-α-mannpyranosides: both lectins accommodate these substrates well. In contrast to the *Dioclea* lectin, concanavalin A binds the 2-*O*-ethyl-substituted ligand significantly better than the unsubstituted monosaccharide. This enhanced binding energy may arise from productive occupation of the pocket adjacent to C2 of the ligand by the *O*-ethyl substituent. This enhancement is not observed for the lectin from *Dioclea* since the pocket does not exist with a glycine at position 226. This reasoning, however, fails to explain the affinity of concanavalin A for the 2-*O*-benzyl-α-mannopyranoide, which should exceed the available space. Alternatively, large hydrophobic ligands such as the 2-*O*-benzyl substituted monosaccharide, may bind distal from the monosaccharide site, utilizing instead the well characterized hydrophobic binding site.[35]

Finally, we note that the C2-alkylated ligands show thermodynamic parameters markedly different from

Table 1. Binding constants and experimental conditions for ligand binding experiments

	Concanavalin A			*Dioclea*		
Ligand	[ligand] (mM)	[lectin] (mM)	K_{eq} (M^{-1})	[ligand] (mM)	[lectin] (mM)	K_{eq} (M^{-1})
1	26.0	0.37	7.6×10^3	20	0.33	3.6×10^3
2	20.0	0.27	6.2×10^3	24	0.27	9.5×10^3
3	20.0	0.3	3.8×10^4	20	0.15	7.1×10^3
4	28.0	0.27	8.3×10^3	11	0.15	1.7×10^4
5	11.0	0.3	2.3×10^4	20	0.19	7.5×10^3
6	20.0	0.3	4.4×10^3	20	0.45	2.7×10^3
7	20.0	0.3	NB[a]	20	0.28	NB[a]
8	20.0	0.29	3.2×10^3	20.0	0.28	3.4×10^3
9	20.0	0.29	NB[a]	20.0	0.28	NB[a]
10	20.0	0.29	NB[a]	20	0.29	NB[a]
11	20.0	0.34	NB[a]	20	0.25	NB[a]
12	20.6	0.32	3.0×10^4	8.7	0.21	1.0×10^4
13	20.0	0.36	6.1×10^3	14.6	0.26	5.3×10^3
14	12.0	0.30	1.9×10^4	12	0.3	1.9×10^4
15	10.0	0.3	NB[a]	10.0	0.3	NB[a]
16	20.0	0.29	NB[a]	20	0.28	NB[a]

[a]No binding observed.

Table 2. Thermodynamic paramaters for ligand binding (kcal mol^{-1}, as °C)

	Concanavalin A			*Dioclea*		
Ligand	ΔG^b	ΔH^c	$T\Delta S$	ΔG^b	ΔH^c	$T\Delta S$
1	−5.3	−6.8	−1.5	−4.8	−7.8	−2.9
2	−5.2	−4.8	+0.4	−5.4	−5.0	+0.4
3	−6.2	−2.8	+3.4	−5.3	−2.4	+2.9
4	−5.4	−4.2	+1.2	−5.8	−3.3	+2.5
5	−6.0	−6.2	−0.2	−5.3	−7.8	−2.5
6	−5.0	−5.3	−0.3	−4.7	−6.1	−1.4
7	NB[5]			NB[d]		
8	−4.8	−6.8	−2.0	−4.8	−6.0	−1.2
9	NB[d]			NB[d]		
10	NB[d]			NB[d]		
11	NB[d]			NB[d]		
12[a]	−6.0	−7.4	−1.4	−5.5	−11.4	−5.1
13[a]	−5.3	−6.9	−1.6	−5.1	−8.6	−3.6
14	−5.8	−7.5	−1.7	−5.6	−9.1	−3.5
15	NB[d]			NB[d]		
16	NB[d]			NB[d]		

[a]Data from ref. 15.
[b]Errors in free energy are in all cases ≤2% of reported values.
[c]Errors in enthalpies are in all cases ≤5% of reported values.
[d]No binding detected.

those for other protein carbohydrate complexes. Thus, the binding of ligands **2–5** proceed with favorable entropic contributions, in contrast to the binding of other legume lectin ligands.[36] A molecular interpretation of this behavior is impossible without additional data, notably ΔC_p and the thermodynamic solvent isotope effect, but may signal a change in the molecular basis of binding.

3-, 4-, and 6-*O*-substituted ligands

The C-4 and C-6 methoxy-substituted ligands **10** and **11** fail to bind either lectins, in agreement with the so-called 'Goldstein rules'.[26] Alternatively, in contrast to the generally accepted view that concanavalin A binds only manno- or glucopyranosides with free hydroxyls at C3, C4 and C6, 3-*O*-methyl-α-mannopyranoside is bound by both lectins with essentially the same affinity as the unsubstituted ligand. This observation is consistent with simple modelling studies, based on the crystallographic structure of the concanavalin A – methyl α-mannpyranoside structure (Fig. 2). The C3-*O*-methyl substitutent of the ligand apparently encounters no disadvantageous steric interactions, and is simply directed into solvent. Similarly, the 2,3-di-*O*-methyl-α-mannopyranoside is bound effectively by both proteins. In contrast, neither the 3-*O*-ethyl- or 2,3-di-*O*-ethyl-α-mannpyranosides are accepted as a ligands by either lectin. The reasoning for this failure to bind is unclear. Whatever hydrogen-bonding requirements exist should be equally well satisfied by both 3-*O*-methyl- and 3-*O*-ethyl-substituted ligands, and the lack of binding presumably has steric origins. While it appears that the ethyl group could be directed into bulk solvent, it may encounter unfavorable interactions with any of the surrounding sidechains (Gly224, Thr226, Arg228, Leu229).

O-Alkyl disaccharides

Both concanavalin A and the lectin from *Dioclea* bind the 1→2, 1→3, 1→4 and 1→6 disaccharides with approximately the same affinity as simple α-mannopyranosides. We have interpreted this behavior in terms of a single high-affinity site binding site that binds the non-reducing terminus of all disaccharides, with little energetic contribution from the reducing terminal mannose to the overall stabilization of the complex.[15] The recent structure of the concanavalin A-trimannoside complex shows several contacts between the central mannose of the trisaccharide and the protein.[29] We note, however, that the mere *existence* of these contacts provides no information regarding the contributions of these contacts to the binding energy.

The binding behavior of the methylated disaccharide methyl 3-*O*-(3-*O*-methyl-α-mannopyranosyl)-α-mannopyranoside is consistent both with our model and the structure of the concanavalin A - methyl 3,6-di-*O*-(α-mannopyranosyl)-α-mannopyranoside complex. In this structure, the C3-hydroxyl of the 1→3 arm of the trimannoside acts as both a hydrogen bond donor and acceptor: clearly, methylation of this hydroxyl should adversely affect binding, were the 1→3 disaccharide bound in the same site as the 1→3 arm of the trimannoside. Similarly, the lack of binding of 6-*O*-(6-*O*-methyl-α-mannopyranosyl)-α-mannopyranoside suggests that the non-reducing terminus of the 1→6 disaccharide binds in a site with similar structural requirements as the monosaccharide binding site.

The binding behavior of 6-*O*-(3-*O*-methyl-α-mannopyranosyl)-α-mannopyranoside is more difficult to explain in terms of a single high-affinity site. If the non-reducing terminus of the disaccharide is bound in the monosaccharide binding site, the 3-*O*-methyl substituent should be tolerated by the lectin. There are two possible explanations for the failure of this ligand to bind. First, the 3-*O*-alkylated disaccharide may bind in a site other than the crystallographically identified site. We have previously raised this possibility based on binding experiments with unnatural bivalent ligands.[37] Alternatively, there is at this time no structural basis for this assertion, and thus Occam's razor dictates a simpler explanation. It is possible that while the *O*-alkylated ligands are bound in the crystallographically identified monsaccharide binding site, they are bound in a slightly different conformation than are native ligands, providing steric constraints not obvious in the binding of monosaccharides. Given the weak binding characteristic of protein–carbohydrate complexes, such mobility within the site does not seem unreasonable.

In conclusion, binding data for a series of methylated monosaccharides support our model of a single, high-affinity binding site that accommodates the monosaccharide and the reducing terminus of all disaccharides. Furthermore, the single difference in amino acid sequence proximal to the monosaccharide binding site provides an opportunity to distinguish the two closely

related proteins by at least 1 kcal mol^{-1}. Finally, our results indicate that structural perturbations to the ligand can affect the structure of the complex in ways unpredictable from crystallographic data, and point to

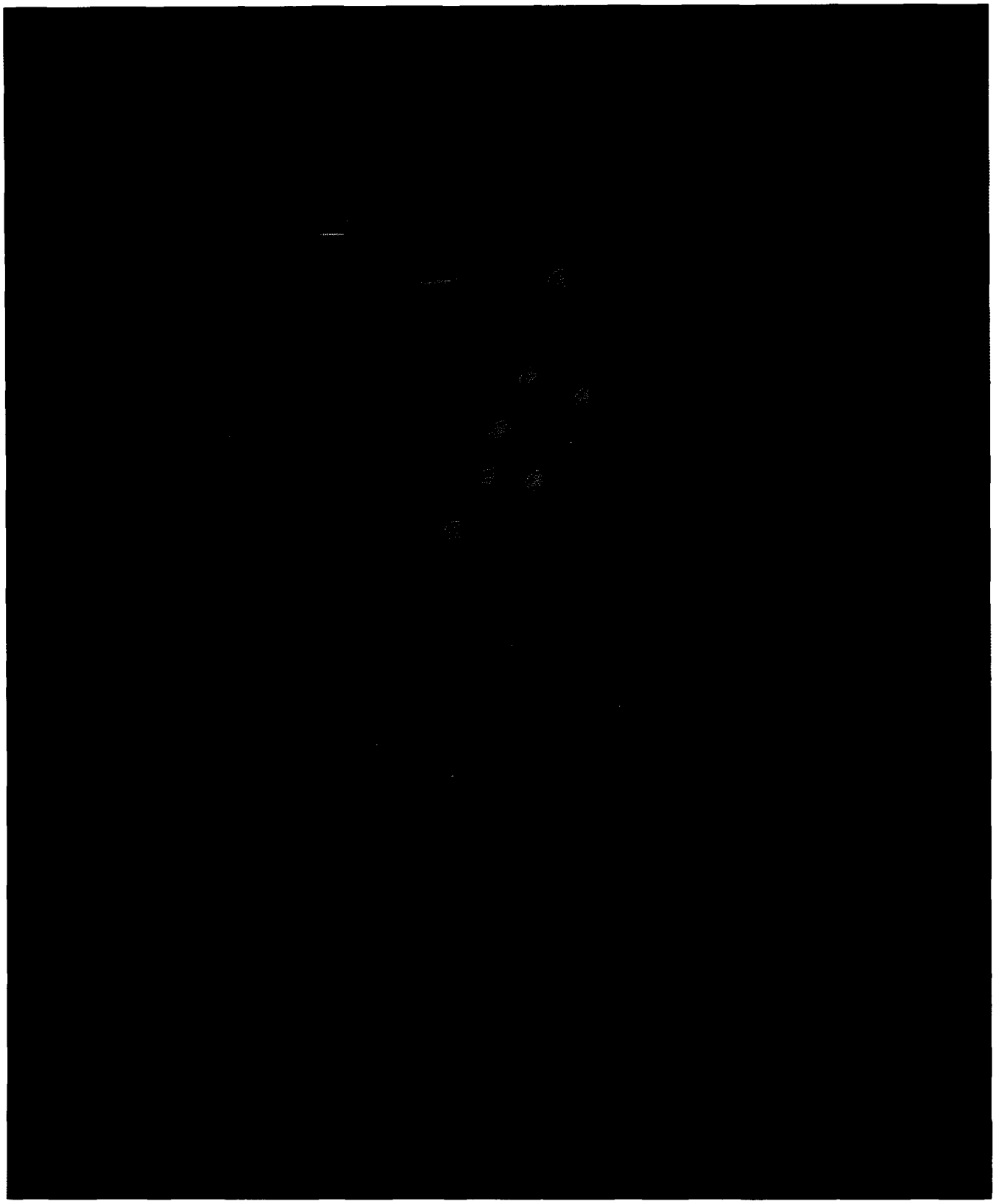


Figure 2. Rendered image of the concanavalin A–methyl α-mannopyranoside complex (from ref. 28). The C2 hydroxyl residue is directed towards Thr226: this residue is replaced by a glycine in the lectin from *Dioclea*. Note the C3 *O*-methyl substitutent is directed towards bulk solvent.

Table 3. Free energy of binding versus C2 substituent volume

		Canavalia	*Dioclea*
Substituent	Volume (Å^3)	ΔG (kcal mol^{-1})	ΔG (kcal mol^{-1})
OH	2.71×10^3	−5.23	−5.21
OMe	2.81×10^4	−5.17	−5.42
OEt	1.03×10^5	−6.23	−5.26
OPr	2.54×10^5	−5.35	−5.37
OBn	9.63×10^5	−5.95	−5.30

the dangers of interpreting binding data for unnatural ligands in terms of crystallographic data on the native complex.

Experimental

General methods

D-Mannose and methyl α-D-mannopyranoside were purchased from Aldrich Chemical Company (Milwaukee, WI) and used without further purification. Tetrahydrofuran was distilled from sodium and benzophenone prior to use. Pyridine and dichloromethane were distilled from calcium hydride prior to use. Acetonitrile was distilled from phosphorous pentoxide prior to use. Benzaldehyde was distilled under reduced pressure immediately prior to use. *N*,*N*-Dimethylformamide was stirred over activated 4 Å molecular sieves and distilled at red. pres. prior to use. Benzyl and allyl bromide was purified by filtration through neutral alumina. Sodium hydride was purchased from Aldrich as a 60% dispersion in mineral oil and washed free of mineral oil with pentane prior to use. All other chemicals were reagent grade and used without further purification. Thin layer chromatography was performed on silica gel 60 F_{254} (Merck) with detection by charring with 5% sulfuric acid in methanol. Column chromatography was performed on flash grade silica gel. ^{1}H NMR were recorded in deuterochloroform or deuterium oxide using a Varian XL-300 instrument operating at 299.925 MHz, and referenced to $CHCl_3$ at 7.24 ppm or HOD at 4.64. ^{13}C NMR spectra were recorded in deuterochloroform or deuterium oxide using a Varian XL-300 instrument operating at 75.427 MHz and referenced to $CHCl_3$ at 77.2 or dioxane at 67.6 ppm. FAB high resolution mass spectral data were collected on a JEOL JMS-SX-102 high resolution mass spectrometer. Melting points were taken with a Uni-Melt capillary melting point apparatus and are uncorrected.

Methyl 3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (19). A soln of 4,6-*O*-benzylidene-α-mannopyranoside[38] (2.40 g, 8.51 mmol) and dibutyl tin oxide (2.12 g, 8.51 mmol) in benzene was refluxed under Dean–Stark conditions for 4 h, then concd. The oily residue was dissolved in dry dimethylformamide (50 mL) and freshly distilled allyl bromide (3.35 g, 28 mmol) was added. The reaction mixture was heated to 100 °C for 45 min, then concd in vacuo. The crude material was purified by silica chromatography (3:1 petroleum ether:ethyl acetate with an increasing ethyl acetate gradient) to yield **19** (2.17 g, 79% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$): δ 2.30 (d, 1H, J=9 Hz, exchangeable, OH-3), 3.39 (s, 3H, OCH_3), 3.75 (dd, 1H, J=1.5 Hz, $J_{2,3}$=3.6 Hz, H-2), 3.76–3.90 (m, 3H), 4.03–4.10 (m, 1H, H-3), 4.13–4.30 (m, 3H), 4.77 (d, 1H, $J_{1,2}$=1.5 Hz, H-1), 5.23–5.38 (m, 2H, ABX system, CH_2=CH), 5.58 (s, 1H, Ph—CH), 5.88–6.00 (m, 1H, CH_2=CH), 7.30–7.52 (m, 5H, aromatic H).

Methyl 3-*O*-allyl-2-*O*-methyl-4,6-*O*-benzylidene-α-D-mannopyranoside. A soln of **19** (1.49 g, 4.6 mmol) and iodomethane (722 mg, 5.1 mmol) in dry THF (30 mL) was added dropwise with stirring to sodium hydride (204 mg, 5.1 mmol) at 0 °C. The reaction mixture warmed to 25 °C and strried for 10 h, then quenched with methanol (2 mL) and extracted with chloroform (3 × 50 mL). The organic extract was washed with brine (2 × 50 mL), dried ($MgSO_4$), concd, and purified by silica chromatography (6:1 petroleum ether:ethyl acetate) to yield methyl 3-*O*-allyl-2-*O*-methyl-4,6-*O*-benzylidene-α-D-mannopyranoside as a pale yellow oil (950 mg, 63% yield): ^{1}H NMR ($CDCl_3$): δ 3.40 (s, 3H), 3.54 (s, 3H), 3.81–3.86 (m 3H), 4.04–4.31 (m, 6H), 4.78 (d, 1H, J=1.27 Hz), 5.60 (s, 1H), 5.86–5.98 (m, 1H), 7.35–7.51 (m, 5H).

Methyl 2-*O*-methyl-4,6-*O*-benzylidene-α-D-mannopyranoside. A soln of methyl 3-*O*-allyl-2-*O*-methyl-4,6-*O*-benzylidene-α-D-mannopyranoside (950 mg, 2.8 mmol), bis-triphenylphosphine rhodium(II)chloride (262 mg, 0.3 mmol, 0.1 eq), and 1,4-diazobicyclo[2.2.2]octane (79.4 mg, 0.7 mmol) in ethanol:benzene:water (7:3:1, 50 mL) was refluxed for 24 h. Solvent was removed under red. pres., and the residue was dissolved in acetone (20 mL) containing mercuric oxide (20 mg 0.1 mmol). Mercuric chloride (600 mg 2.2 mmol) in acetone:water (9:1, 10 mL) was added and the reaction mixture was stirred at 25 °C for 5 h. Solvent was removed under red. pres. and the residue extracted with chloroform (2 × 100 mL). The organic extract was washed with 30% aq KBr soln (2 × 50 mL), dried ($MgSO_4$), concd, and purified by silica gel chromatography (ethyl acetate) to yield methyl 2-*O*-methyl-4,6-*O*-benzylidene-α-D-mannopyranoside as a pale yellow oil (600 mg, 72% yield): ^{1}H NMR ($CDCl_3$) δ 2.61 (s, OH exchangeable), 3.40 (s, 3H), 3.54 (s, 3H), 3.57–3.58 (m, 1H), 3.78–3.86 (m, 3H), 4.04–4.08 (m, 1H), 4.24–4.28 (m, 1H), 4.81 (s, 1H), 5.56 (s, 1H), 7.35–7.51 (m, 5H).

Methyl 2-*O*-methyl-α-D-mannopyranoside (2). A soln of methyl 2-*O*-methyl-4,6-*O*-benzylidene-α-D-mannopyranoside (600 mg, 2.0 mmol) in 60% aq acetic acid (20 mL) was heated to 80 °C for 3 h. Solvent was removed under red. pres. The residue was extracted with deionized water (3 × 10 mL) and concd. The crude material was purified by silica gel chromatography (4:1 ethyl acetate:methanol) to yield **2** as colorless glass (360 mg, 85% yield): ^{1}H NMR (D_2O): δ 3.38 (s, 3H, 1-*O*-CH_3), 3.43 (s, 3H, 2-O-CH_3), 3.65–3.71 (m, 1H, C-4), 3.75–3.78 (m, 1H, C-5), 3.84–3.88 (d, 2H, C-6,

C-6′, J=1.89 Hz), 4.90 (s, 1H, C-1); ^{13}C NMR ($CDCl_3$): δ 54.7 (OCH_3), 58.8 (2-O-CH_3), 60.9 (C-6, C-6′), 67.1, 70.2 (C-5), 72.4 (C-4), 79.8, 97.6 (C-1); HRMS calcd for $C_8H_{17}O_6$ ($M+H^+$) 209.1025; found 209.1044.

Methyl 2-*O*-ethyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside. A soln of **19** (630 mg, 2.0 mmol) and iodoethane (335 mg, 2.2 mmol) in dry THF (20 mL) was added dropwise with stirring to sodium hydride (86 mg, 2.2 mmol) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 20 h then quenched with methanol (4 mL) and extracted with dichloromethane (2×50 mL). The organic extract was dried ($MgSO_4$) concentrated and purified by silica gel chromtography (6:1 petroleum ether:ethyl acetate) to yield methyl 2-*O*-ethyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside as a pale yellow oil (170 mg, 25% yield): ^{1}H NMR ($CDCl_3$): δ 1.26 (t, 3H, J=7.5 Hz), 3.35 (s, 3H), 3.62–3.92 (m, 6H), 4.09–4.40 (m, 4H), 4.71 (s, 1H), 5.10–5.40 (m, 2H), 5.60 (s, 1H), 5.85–6.00 (m, 1H), 7.30–7.56 (m, 5H) ppm.

Methyl 2-*O*-ethyl-4,6-*O*-benzylidene-α-D-mannopyranoside. A soln of methyl 2-*O*-ethyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (170 mg 0.5 mmol), tris(triphenylphosphine)rhodium chloride (45 mg, 0.05 mmol), and 1,4-diazabicyclo[2.2.2]octane (14 mg, 0.12 mmol) in ethanol:benzene:water (7:3:1, 11 mL) was refluxed for 24 h. Solvent was removed under red. pres., and the residue dissolved in acetone (10 mL) containing mercuric oxide (20 mg, 0.1 mmol). To this soln was added mercuric chloride (300 mg, 0.6 mmol) in acetone:water (9:1, 5 mL), and the reaction mixture was stirred at 25 °C for 10 h. Sovent was removed under red. pres., and the residue was extracted with dichloromethane (2×50 mL). The organic phase was washed with 30% aq KBr (1×50 mL), dried ($MgSO_4$), concd and purified by silica gel chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 2-*O*-ethyl-4,6-*O*-benzylidene-α-D-mannopyranoside as a pale yellow oil (100 mg, 66% yield): ^{1}H NMR ($CDCl_3$): δ 1.26 (t, 3H, J=7.5 Hz), 2.08 (s, OH exchangeable), 3.40 (s, 3H), 3.55–3.87 (m, 6H), 3.98–4.30 (m, 2H), 4.80 (s, 1H), 5.60 (s, 1H), 7.30–7.55 (m, 5H).

Methyl 2-*O*-ethyl-α-D-mannopyranoside (3). A soln of methyl 2-*O*-ethyl-4,6-*O*-benzylidene-α-D-mannopyranoside (100 mg, 0.3 mmol) in 60% aqueous acetic acid (20 mL) was heated to 70 °C for 2 h. Solvent was removed under red. pres. and the crude material purified by silica gel chromatography (ethyl acetate) to yield *3* as a pale yellow oil (37 mg, 52% yield): ^{1}H NMR (D_2O): δ 1.10 (t, 3H, J=7.5 Hz), 3.32 (s, 3H), 3.47–3.90 (m, 8H), 4.78 (s, 1H); ^{13}C NMR (D_2O): δ 14.3 (OCH_2CH_3), 54.6 (OCH_3), 60.8 (OCH_2CH_3), 67.0, 67.5 (C-6), 70.2, 72.3, 77.9, 98.5 (C-1); HRMS calcd for $C_9H_{19}O_6$ ($M+H^+$) 223.1181; found 223.1179.

Methyl 2-*O*-propyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside. A soln of **19** (1.00 g, 3.1 mmol) and iodopropane (581 mg, 3.4 mmol) in dry THF (20 mL) was added dropwise with stirring to sodium hydride (137 mg, 3.4 mmol) at 0 °C. The solution was warmed to 25 °C and stirred for 24 h. The reaction mixture was quenched with methanol (5 mL) and extracted with dichloromethane (2×50 mL). The organic extract was washed with 1 M HCl (1×30 mL) and brine (2×30 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica gel chromatography (6:1 petroleum ether:ethyl acetate) to yield methyl 2-*O*-propyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside as a pale yellow oil (50 mg, 5% yield): ^{1}H NMR ($CDCl_3$): δ 0.953 (t, 3H, J=7.25 Hz), 1.80 (dq, 2H, J_1=6.97 Hz, J_2=7.25 Hz), 3.40 (s, 3H), 3.53–3.92 (m, 7H), 4.06–4.38 (m, 3H), 4.61 (s, 1H), 5.15 (dd, 1H, $J_{1,2}=J_{3,4}$=1.71, $J_{1,3}=J_{2,4}$=10.4), 5.30 (dd, 1H, J_1=1.71 Hz, J_2=17.3 Hz), 5.60 (s, 1H), 5.82–6.00 (m, 1H), 7.32–7.52 (m, 5H).

Methyl 4,6-*O*-benzylidene-2-*O*-propyl-α-D-mannopyranoside. A soln of methyl 2-*O*-propyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (50 mg, 0.14 mmol), tris(triphenylphosphine)rhodium chloride (13 mg, 0.01 mmol) and 1,4-diazabicyclo[2.2.2]octane (4 mg, 0.03 mmol) in ethanol:benzene:water (7:3:1, 11 mL) was refluxed for 24 h. The solvent was removed under red. pres. and the residue dissolved in acetone (8 mL) containing mercuric oxide (20 mg, 0.1 mmol). To this soln was added a soln of mercuric chloride (240 mg, 0.9 mmol) in acetone:water (9:1, 4 mL), and the resulting soln was stirred at 25 °C for 2 h. Solvent was removed under red. pres. and the residue was extracted with dichloromethane (3×25 mL) then washed with 30% aq KBr (2×25 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica gel chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 4,6-*O*-benzylidene-2-*O*-propyl-α-D-mannopyranoside as a pale yellow oil (30 mg, 65% yield): ^{1}H NMR ($CDCl_3$): δ 0.949 (t, 3H, J=7.52 Hz), 1.65 (dq, 2H, J_1=6.83, J_2=7.52 Hz), 2.39 (d, 1H, J=9.03 Hz), 3.40 (s, 3H), 3.46–3.86 (m, 6H), 3.99–4.28 (s, 1H), 5.58 (s, 1H), 7.30–7.55 (m, 5H).

Methyl 2-*O*-propyl-α-D-mannopyranoside (4). A soln of methyl 4,6-*O*-benzylidene 2-*O*-propyl-α-D-mannopyranoside (30 mg, 0.1 mmol) in 60% aq acetic acid (10 mL) was heated at 70 °C for 90 min. Solvent was removed under red. pres. and the crude product purified by silica gel chromatography (4:1 ethyl acetate:methanol) to yield **4** as a pale yellow oil (11 mg, 63% yield): ^{1}H NMR (D_2O): δ 0.818 (t, 3H, J=7.32 Hz), 1.52 (dq, 2H, $J_1=J_2$=7.32 Hz), 3.33 (s, 3H), 3.48–3.90 (m, 9H), 4.82 (s, 1H); ^{13}C NMR (D_2O): δ 23.2, 54.6, 60.8, 67.0, 70.4, 72.4, 73.8, 78.1, 98.6; HRMS calcd for $C_{10}H_{21}O_6$ ($M+H^+$) 237.1338, found 237.1338.

Methyl 2-*O*-benzyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside. A soln of **19** (2.17 g, 6.7 mmol), benzyl bromide (1.21 g, 7.1 mmol) and tetrabutylammonium bromide (217 mg, 0.7 mmol) in dry THF (15 mL) was added dropwise with stirring to sodium hydride (405 mg, 10.1 mmol) at 0 °C. The reaction was warmed to 25 °C and stirred for 12 h. The reaction was quenched with methanol (2 mL) and extracted with

methylene chloride (3 × 20 mL). The organic extract was washed with water (2 × 100 mL) and brine (2 × 100 mL), dried ($MgSO_4$), and concd. The crude product was purified by silica chromatography (6:1 petroleum ether:ethyl acetate) to yield methyl 2-*O*-benzyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside as a colorless glass (1.81 g, 65% yield): ^{1}H NMR ($CDCl_3$): δ 2.30 (d, 1H, J=9 Hz, exchangeable, OH-3), 3.39 (s, 3H, OCH_3), 3.75 (dd, 1H, J_1=1.5 Hz, J_2=3.6 Hz, H-2), 3.76–3.90 (m, 3H), 4.03–4.10 (m, 1H, H-3), 4.13–4.30 (m, 3H), 4.77 (d, 1H, $J_{1,2}$=1.5 Hz, H-1), 5.23–5.38 (m, 2H, ABX system, $C\underline{H}_2$=CH), 5.58 (s, 1H, Ph-C$\underline{H}$), 5.88–6.00 (m, 1H, CH_2=C$\underline{H}$), 7.30–7.52 (m, 10H, aromatic H).

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (24). A soln of methyl 2-*O*-benzyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (1.81 g, 4.4 mmol), tris(triphenylphosphine)rhodium(I)chloride (407 mg, 0.4 mmol), and 1,8 diazabicyclo[2.2.2]octane (128 mg, 1.1 mmol) in ethanol:water:benzene (7:3:1, 30 mL) was refluxed for 24 h. Solvent was removed under red. pres. and the residue dissolved in acetone (30 mL) containing mercuric oxide (20 mg, 0.1 mmol). To this soln was added mercuric chloride (300 mg, 0.6 mmol) in acetone:water (9:1, 15 mL), and the reaction mixture stirred at 25 °C for 18 h. Solvent was removed under red. pres. and the residue was extracted with methylene chloride (3 × 50 mL). The organic extract was washed with 30% aq potassium bromide (2 × 100 mL) and water (2 × 100 mL), dried ($MgSO_4$), and concd. The crude product was purified by silica chromoatography (9:1 toluene:ethyl acetate) to yield **24** a pale yellow oil (1.25 g, 77% yield): ^{1}H NMR ($CDCl_3$): δ 2.30 (d, 1H, J=9 Hz, exchangeable, OH-3), 3.39 (s, 3H, OCH_3), 3.75 (dd, 1H, $J_{1,2}$ =1.5 Hz, $J_{2,3}$=3.6 Hz, H-2), 3.76–3.90 (m, 3H), 4.03–4.10 (m, 1H, H-3), 4.13–4.30 (m, 3H), 4.77 (d, 1H, $J_{1,2}$=1.5 Hz, H-1), 5.58 (s, 1H, Ph-C$\underline{H}$), 7.30–7.52 (m, 10H, aromatic H).

Methyl 2-*O*-benzyl-α-D-mannopyranoside (5). A soln of **24** (570 mg, 1.4 mmol) in 60% aq acetic acid (20 mL) was heated to 80 °C for 1 h. Solvent was removed under red. pres., and the crude material purified by silica gel chromatography (4:1 ethyl acetate:methanol) to yield **5** as pale yellow crystals (30 mg, 10% yield): ^{1}H NMR (D_2O): δ 3.35 (s, OCH_3), 3.50–3.88 (m, 6H), 4.60–4.75 (m, 3H), 7.30–7.50 (m, 5H); ^{13}C NMR (D_2O) 55.6 (OCH_3), 67.0 (C-6), 70.4, 72.4, 73.4, 77.4, 98.5, 128.6, 128.7, 128.8; HRMS calcd for $C_{14}H_{21}O_6$ ($M+H^+$) 285.1338; found 285.1342.

Methyl 3-*O*-methyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside. A suspension of **18** (1.21 g, 4.3 mmol) and dibutyltin oxide (1.07 g, 4.3 mmol) in benzene (50 mL) was heated under Dean–Stark conditions for 12 h. The solvent was removed by distillation and iodomethane (609 mg, 4.2 mmol) in dry DMF (20 mL) was added. The soln was heated at reflux for 4 h, then solvent was removed in vacuo, and the crude material purified by silica chromatography (4:1 petroleum ether:ethyl acetate with increasing ethyl acetate gradient) to yield methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside as a pale yellow oil (650 mg, 51% yield): ^{1}H NMR ($CDCl_3$): δ 2.70 (s, OH exchangeable); 3.41 (s, 3H); 3.58 (s, 3H); 3.65–4.35 (m, 6H); 4.81 (s, 1H); 5.60 (s, 1H); 7.34–7.59 (m, 5H).

Methyl 3-*O*-methyl-α-D-mannopyranoside (6). A soln of methyl 3-*O*-methyl-4,6-*O*-benzylidene-α-D-mannopyranoside (650 mg, 2.2 mmol) in 60% aq acetic acid (20 mL) was heated to 80 °C with stirring for 30 min. Solvent was removed from the cooled reaction mixture and the crude material purified by silica chromatography (4:1 ethyl acetate:methanol) to yield **6** as a colorless glass (317 mg, 69% yield): ^{1}H NMR (D_2O): δ 3.34 (s, 3H); 3.35 (s, 3H); 3.50–3.87 (m, 6H); 4.73 (s, 1H); ^{13}C NMR (D_2O): δ 53.9 (s, OCH_3); 55,4 (s, 3-O-CH_3); 60.1; 60.9; 64.7; 71.7; 79.2; 100.0 (C-1); HRMS calcd for $C_8H_{17}O_6$ ($M+H^+$) 209.1025, found 209.1022.

Methyl 4,6-*O*-benzylidene-3-*O*-ethyl-α-D-mannopyranoside. A suspension of **18** (500 mg, 1.77 mmol) and dibutyltin oxide (441 mg, 1.77 mmol) in benzene (50 mL) was refluxed under Dean–Stark conditions for 12 h. Solvent was removed by distillation and iodoethane (277 mg, 1.77 mmol) in dry DMF (15 mL) was added. The reaction mixture was refluxed for 8 h. Solvent was removed under red. pres. to yield an orange oil that was purified by silica chromatography (3:1 petroleum ether:ethyl acetate with increasing ethyl acetate gradient) to yield methyl 4,6-*O*-benzylidene-3-*O*-ethyl-α-D-mannopyranoside as a pale yellow oil (120 mg, 22% yield): ^{1}H NMR ($CDCl_3$): δ 1.81 (t, 3H, J=7.5 Hz); 3.40 (s, 3H); 3.65–3.82 (m, 5H); 3.95–4.13 (m, 2H); 4.24–4.34 (m, 1H); 4.80 (s, 1H); 5.60 (s, 1H); 7.33–7.55 (m, 5H).

Methyl 3-*O*-ethyl-α-D-mannopyranoside (7). A soln of methyl 4,6-*O*-benzylidene-3-*O*-ethyl-α-D-mannopyranoside (120 mg, 0.4 mmol) in 60% aq acetic acid (10 mL) was heated to 80 °C with stirring for 30 min. The solvent was removed under red. pres. and the crude material was purified by silica chromatography (4:1 ethyl acetate:methanol) to yield an amorphous white solid (76 mg, 88%): ^{1}H NMR (D_2O): δ 1.11 (t, 3H, J=7.5 Hz); 3.30 (s, 3H); 3.39–3.82 (m, 7H); 4.02 (s, 1H); 4.69 (s, 1H); ^{13}C NMR (D_2O): δ 17.0 (s, 3-O-$CH_2\underline{C}H_3$); 57.4 (s, OCH_3); 63.6 (3-O-$C\underline{H}_2CH_3$); 67.6 (C-6); 68.3; 69.1; 75.2; 80.9; 103.4 (C-1); $C_9H_{19}O_6$ ($M+H^+$) 223.1181, found 223.1200.

Methyl 2,3-di-*O*-methyl-4,6-*O*-benzylidene-α-D-mannopyranoside. A solution of methyl 4,6-*O*-benzylidene-α-mannopyranoside (100 mg, 0.4 mmol) in dry tetrahydrofuran (10 mL) was added dropwise with stirring to sodium hydride (28.4 mg, 0.7 mmol) under argon at 0 °C. The reaction mixture warmed to 25 °C and stirred for 8 h. The reaction mixture was quenched with methanol (1 mL) and extracted with methylene chloride (25 mL × 2). The organic extract was washed with brine (2 × 30 mL), dried ($MgSO_4$), and concd. The

crude material was purified by silica chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 2,3-di-*O*-methyl-4,6-*O*-benzylidene-α-D-mannopyranoside as a colorless oil (20 mg, 18% yield): ^{1}H NMR ($CDCl_3$): δ 3.40 (s, 3H); 3.56 (s, 6H); 3.65–3.91 (m, 3H); 4.04–4.16 (m, 1H); 4.22–4.31 (m, 1H); 5.60 (s, 1H); 7.32–7.55 (m, 5H).

Methyl 2,3-di-*O*-methyl-α-D-mannopyranoside (8). A soln of methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-mannopyranoside (20 mg, 0.06 mmol) in 60% aq acetic acid (10 mL) was heated with stirring to 80 °C for 30 min. Solvent was removed under red. pres. and the crude material purified by silica chromatography (4:1 ethyl acetate:methanol with increasing methanol gradient) to yield **8** as a colorless glass (14 mg, 100% yield): ^{1}H NMR (D_2O): δ 3.36 (s, 3H); 3.40 (s, 6H); 3.51–3.56 (m, 2H); 3.61–3.69 (m, 2H); 3.75–3.86 (m, 2H): 4.91 (s, 1H); ^{13}C NMR (D_2O): δ 54.1 (OCH_3); 56.5 (3-O-CH_3); 57.0 (2-O-CH_3); 60.1 (C-6); 65.0; 72.3; 74.6; 79.8; 96.8 (C-1).

Methyl 2,3-di-*O*-ethyl-4,6-*O*-benzylidene-α-D-mannopyranoside. A soln of methyl 4,6-*O*-benzylidene-α-mannopyranoside (500 mg, 1.8 mmol) in dry tetrahydrofuran (15 mL) was added to sodium hydride (141.6 mg, 3.54 mmol) at 0 °C with stirring under argon. To this reaction mixture, iodoethane (552. mg, 3.6 mmol) was added. The reaction mixture was warmed to 25 °C and stirred for 8 h. The reaction mixture was quenched with methanol (1 mL) and extracted with methylene chloride (2 × 25 mL). The organic extract was neutralized with 1 M HCl and washed with brine (2 × 50 mL). The organic extract was dried ($MgSO_4$) and concd. The crude material was purified by silica gel chromatography (6:1 petroleum ether:ethyl acetate) to yield methyl 2,3-di-*O*-ethyl-4,6-*O*-benzylidene-α-D-mannopyranoside as a clear, colorless oil (60 mg, 10% yield): ^{1}H NMR ($CDCl_3$): δ 1.10–1.25 (m, 6H); 3.40 (s, 3H); 3.48–3.87 (m, 6H); 4.01–4.35 (m, 4H); 4.71 (s, 1H); 5.60 (s, 1H); 7.32–7.55 (m, 5H).

Methyl 2,3-di-*O*-ethyl-α-D-mannopyranoside (9). A soln of methyl 4,6-*O*-benzylidene-2,3-di-*O*-ethyl-α-D-mannopyranoside (60 mg, 0.18 mmol) in 60% aq acetic acid (5 mL) was heated with stirring to 80 °C for 30 min. Solvent was removed from the cooled reaction mixture under red. pres. The crude product was purified by column chromatography (ethyl acetate) to yield **9** as a colorless glass (7 mg, 16% yield): ^{1}H NMR (D_2O): δ 1.12–1.20 (m, 6H); 3.34 (s, 3H); 3.56–3.87 (m, 10H); 4.64 (s, 1H).

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside. A soln of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (632 mg, 1.6 mmol) and tetrabutylammonium bromide (102 mg, 0.3 mmol) in dry THF (10 mL) was added dropwise with stirring to sodium hydride (76.3 mg, 3.2 mmol). To this reaction mixture was added benzyl bromide (534 mg, 3.2 mmol), and the resulting soln stirred at 25 °C for 10 h. The reaction mixture was quenched with methanol (5 mL) and extracted with methylene chloride (2 × 50 mL). The organic phase was washed with brine (2 × 50 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside as a pale yellow oil (370 mg, 36% yield): ^{1}H NMR ($CDCl_3$): δ 3.35 (s, 3H); 3.73–4.00 (m, 4H); 4.21–4.30 (m, 3H); 4.55–4.86 (m, 4H); 5.64 (s, 1H); 7.29–7.56 (m, 15H).

Methyl 2,3-di-*O*-benzyl-α-D-mannopyranoside. A soln of methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (990 mg, 2.1 mmol) in 60% aq acetic acid (25 mL) was heated to 80 °C with stirring for 45 min. Solvent was removed in vacuo, and the crude material purified by silica chromatography (ethyl acetate) to yield methyl 2,3-di-*O*-benzyl-α-D-mannopyranoside as a colorless glass (470 mg, 59% yield): ^{1}H NMR ($CDCl_3$): δ 3.30 (s, 3H); 3.49–4.03 (m, 6H); 4.39–4.71 (m, 5H); 7.17–7.32 (m, 10H).

Methyl 2,3-di-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside. *t*-Butyldiphenylchlorosilane (206 mg, 0.75 mmol) was added dropwise with stirring to a soln of methyl 2,3-di-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside (280 mg, 0.75 mmol) and imidazole (51 mg, 0.75 mmol) in dry tetrahydrofuran (10 mL). The reaction mixture stirred at 25 °C for 8 h then extracted with methylene chloride (3 × 25 mL). The organic phase was washed with brine (2 × 50 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 2,3-di-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside as a colorless oil (190 mg, 42% yield): ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 9H); 3.30 (s, 3H); 3.60–4.16 (m, 7H); 4.52–4.75 (m, 4H); 7.28–7.80 (m, 20H).

Methyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside. A soln of methyl 2,3-di-*O*-benzyl-6-*O*-*t*-butytldiphenylsilyl-α-D-mannopyranoside (190 mg, 0.3 mmol) in dry tetrahydrofuran (10 mL) was added to sodium hydride (12.4 mg, 0.3 mmol) at 0 °C under argon. The reaction mixture stirred at 0 °C for 10 min then iodomethane (44 mg, 0.3 mmol) was added. The solution was warmed to 25 °C and stirred for 8 h. The reaction mixture was quenched with methanol (1 mL) and extracted with methylene chloride (3 × 25 mL). The organic phase was washed with brine (2 × 50 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica chromatography (6:1 petroleum ether:ethyl acetate) to yield methyl 2,3-di-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-4-*O*-methyl-α-D-mannopyranoside as a pale yellow oil (120 mg, 63% yield): ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 9H); 3.30 (s, 3H); 3.48–3.56 (m, 4H); 3.68–3.97 (m, 5H); 4.62–4.80 (m, 5H); 7.31–7.80 (m, 20H).

Methyl 2,3-di-*O*-benzyl-4-*O*-methyl-α-D-mannopyranoside. HF/pyridine (5 mL) was added dropwise with stirring to a soln of methyl 2,3-di-*O*-benzyl-6-*O*-*t*-butyl-

diphenylsilyl-4-*O*-methyl-α-D-mannopyranoside (120 mg, 0.19 mmol) in dry THF (10 mL). The soln was stirred at 25 °C for 8 h then extracted with methylene chloride (3 × 25 mL). The organic phase was washed with brine (2 × 50 mL), dried (MgSO4), and concd. The crude material was purified by silica gel chromatography (ethyl acetate) to yield methyl 2,3-di-*O*-benzyl-4-*O*-methyl-α-D-mannopyranoside as a pale yellow oil (60 mg, 80% yield): ^{1}H NMR ($CDCl_3$): δ 3.30 (s, 3H); 3.45–3.86 (m, 9H); 4.55–4.75 (m, 5H); 7.50–7.62 (m, 10H).

Methyl 4-*O*-methyl-α-D-mannopyranoside (10). Hydrogen gas (1 atm, 25 °C) was bubbled through a suspension of methyl 2,3-di-*O*-benzyl-4-*O*-methyl-α-D-mannopyranoside (60 mg, 0.15 mmol) and 10% Pd/C (50 mg) in methanol (10 mL) for 5 h. The reaction mixture was filtered through Celite, concd, and the crude material purified by silica chromatography (ethyl acetate) to yield **10** as a colorless glass (25 mg, 80% yield): ^{1}H NMR (D_2O): δ 3.30 (s, 3H); 3.44 (s, 3H); 3.64–3.84 (m, 6H); 4.65 (s, 1H); ^{13}C NMR (D_2O): δ 54.4; 56.1; 60.1; 69.0; 69.8; 71.0; 77.0; 100.0; HRMS calcd for $C_8H_{17}O_6$ ($M+H^+$) 209.1025; found 209.1033.

Methyl 6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside. A soln of methyl α-D-mannopyranoside (3.00 g, 15.4 mmol), imidazole (2.31 g, 34 mmol), and *t*-butyldiphenylsilylchloride (4.66 g, 16.9 mmol) in dry THF (75 mL) was heated to 80 °C with stirring for 4 h. The cooled reaction was extracted with methylene chloride. The organic phase was washed with water (2 × 50 mL), dried ($MgSO_4$), and concd. The crude product was purified by silica chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside as a clear glass (5.92 g, 95% yield): ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 9H), 3.38 (s, 3H), 3.59–3.66 (m, 2H), 3.82–3.84 (m, 2H), 3.92–3.95 (m, 2H), 7.35–7.45 (m, 5H), 7.60–7.80 (m, 5H).

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside. A solution of methyl 6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside (5.92 g, 14.6 mmol), tetrabutylammonium iodide (0.54 g, 1.46 mmol), and benzyl bromide (11.26 g, 65.7 mmol) in dry THF (100 mL) was added dropwise under nitrogen at 0 °C to a suspension of sodium hydride (2.63 g, 65.7 mmol). The reaction mixture was warmed to 25 °C and stirred for 10 h then quenched with methanol. The resulting soln was extracted with methylene chloride (3 × 75 mL). The organic phase was washed with water (2 × 50 mL), dried ($MgSO_4$), and concd. The crude product was recrystallized from petroluem ether to yield methyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside as pale yellow crystals (8.68 g, 88% yield): ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 9H), 3.38 (s, 3H), 3.65–4.18 (m, 6H), 4.60–5.00 (m, 7H), 7.20–7.91 (m, 25H).

Methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (20). To a soln of methyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-α-D-mannopyranoside (500 mg, 0.7 mmol) in dry THF (10 mL) was added HF/pyridine (1.47 g, 14.8 mmol). The reaction mixture was stirred at 25 °C for 16 h then extracted with methylene chloride. The organic phase was washed with 1 M HCl (2 × 150 mL), satd with aq $NaHCO_3$ (2 × 150 mL), and water (1 × 150 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica chromatography (4:1 petroluem ether:ethyl acetate) to yield **20** as a colorless oil (300 mg, 93% yield): ^{1}H NMR ($CDCl_3$): δ 3.38 (s,3H), 3.60–4.03 (m, 6H), 4.60–5.00 (m, 7H), 7.20–7.58 (m, 15 H).

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-mannopyranoside. A soln of *20* (100 mg, 0.22 mmol) and iodomethane (31 mg, 0.22 mmol) in dry THF (10 mL) was added dropwise to NaH (8.6 mg, 0.22 mmol). The reaction mixture was stirred at 25 °C for 5 h, then the reaction mixture was quenched with methanol (1 mL) and extracted with methylene chloride (2 × 25 mL). The organic phase was washed with brine (2 × 20 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-mannopyranoside as a pale yellow oil (30 mg, 29% yield): ^{1}H NMR ($CDCl_3$): δ 3.31 (s, 3H); 3.38 (s, 3H), 3.60–3.98 (m, 6H); 4.50–4.63 (m, 3H); 4.70–4.79 (m, 3H); 4.90–4.97 (m, 1H); 7.24–7.42 (m, 15H).

Methyl 6-*O*-methyl-α-D-mannopyranoside (11). Hydrogen gas (1 atm, 25 °C) was bubbled through a suspension of methyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-mannopyranoside (30 mg, 0.06 mmol) and 10% Pd/C (30 mg) in absolute methanol (5 mL) for 4 h. The reaction mixture was filtered through Celite, concd, and the crude product purified by silica chromatography (4:1 ethyl acetate:methanol) to yield **11** as a colorless glass (3.5 mg, 27% yield): ^{1}H NMR (D_2O): δ 3.33 (s, 3H); 3.34 (s, 3H); 3.53–3.73 (m, 6H); 4.67 (s, 1H); ^{13}C NMR (D_2O): δ 54.0 (OCH_3); 56.7 (6-*O*-CH_3); 60.1; 69.0; 69.8; 71.0; 77.0; 100.0 (C-1); HRMS calcd for $C_8H_{17}O_6$ ($M+H^+$) 209.1025; found 209.1030.

Methyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside. Freshly distilled benzaldehyde (40 mL, 361 mmol) was added to a soln of methyl 1-thio-α-D-mannopyranoside (1.10 g, 5.2 mmol) in 96% formic acid (40 mL). The reaction mixture stirred at 25 °C for 15 min then poured into a biphasic mixture of hexane (200 mL) and water (200 mL) containing $NaHCO_3$ (60 g). The resulting white precipitate was collected by vacuum filtration and recrystallized from benzene to yield methyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (140 mg, 9.0% yield): ^{1}H NMR ($CDCl_3$): δ 1.57 (s, OH exchangeable); 2.17 (s, 3H); 3.84–4.31 (m, 6H); 5.26 (s, 1H), 5.58 (s, 1H); 7.35–7.55 (m, 5H).

Methyl 4,6-*O*-benzylidene-3-*O*-methyl-1-thio-α-D-mannopyranoside. A suspension of methyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (140 mg, 0.5 mmol) and dibutyltin oxide (117 mg, 0.5 mmol) in benzene (50

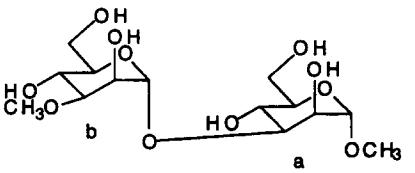
mL) was refluxed under Dean–Stark conditions for 10 h. Solvent was removed and iodomethane (66.7 mg, 0.5 mmol, 1.0 eq) in dry DMF (10 mL) was added. The solution was refluxed for 8 h, then solvent was removed in vacuo to yield a dark yellow oil. The crude material was purified by silica chromatography (3:1 petroleum ether:ethyl acetate with increasing ethyl acetate gradient) to yield methyl 4,6-*O*-benzylidene-3-*O*-methyl-1-thio-α-D-mannopyranoside as a pale yellow oil (30 mg, 21% yield): ^{1}H NMR ($CDCl_3$): δ 1.57 (s, OH exchangeable); 2.17 (s, 3H); 3.55 (s, 3H); 3.84–4.31 (m, 6H); 5.26 (s, 1H), 5.58 (s, 1H); 7.35–7.55 (m, 5H).

Methyl 3-*O*-methyl-1-thio-α-D-mannopyranoside. A soln of methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside (40 mg, 0.13 mmol) in 60% aq acetic acid (10 mL) was heated with stirring to 80 °C for 45 min. Solvent was removed under red. pres. to yield a pale yellow oil. The crude material was purified by silica chromatography (4:1 ethyl acetate:methanol) to yield a clear, colorless glass (20 mg, 70% yield): ^{1}H NMR (D_2O): δ 2.10 (s, 3H); 3.38 (s, 3H); 3.60–4.01 (m. 5H); 4.23 (m, 1H); 5.20 (s, 1H).

Methyl 2-*O*-acetyl-3-*O*-methyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (25). Acetic anhydride (9.8 mg, 0.1 mmol) was added to a soln of methyl 4,6-*O*-benzylidene-3-*O*-methyl-1-thio-α-D-mannopyranoside (30 mg, 0.1 mmol) in dry pyridine (7.6 mg, 0.1 mmol). The reaction mixture was stirred at 25 °C for 2 h then dil with methylene chloride (2 × 25 mL). The soln was washed with 1 M HCl (1 × 25 mL), satd aq $NaHCO_3$ (1 × 25 mL), and water (1 × 25 mL), dried ($MgSO_4$) and concd. The crude material was purified by silica chromatography (8:1 petroleum ether:ethyl acetate) to yield **25** as a colorless glass (20 mg, 59% yield): ^{1}H NMR ($CDCl_3$): δ 2.15 (s, 3H); 2.16 (s, 3H); 3.55 (s, 3H); 3.84–4.31 (m, 6H); 5.26 (s, 1H), 5.58 (s, 1H); 7.35–7.55 (m, 5H).

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-O-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside. *N*-Iodosuccinamide (111 mg, 0.5 mmol) was added to a soln of **25** (80 mg, 0.2 mmol) and **24** (140 mg, 0.4 mmol) in methylene chloride (10 mL). The mixture was stirred with in the dark at 25 °C for 20 min, then cooled to 0 °C. Trifluoromethanesulfonic acid in CH_2Cl_2 (5 mL of 30 mM soln) was added, and the reaction mixture was stirred at 0 °C for 2 h. The soln was dild with methylene chloride (20 mL), filtered through Celite and washed with satd aq $NaHCO_3$ (2 × 25 mL), brine (2 × 25 mL), and 10% aq sodium thiosulfate soln (2 × 25 mL). The organic phase was dried ($MgSO_4$) and concd. The crude material was purified by silica gel chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside as a pale orange oil (140 mg, 92% yield): ^{1}H NMR ($CDCl_3$): δ 2.09 (s, 3H); 3.35 (s, 3H); 3.41 (s, 3H); 3.70–4.31 (m, 13H); 4.69–4.79 (m, 3H); 5.55–5.65 (m, 2H); 7.27–7.54 (m, 15H).

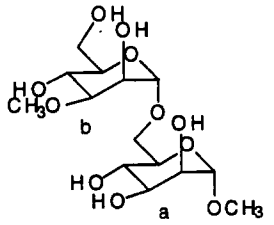
Methyl 3-*O*-(3-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside (14). A suspension of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside (140 mg, 0.2 mmol) and 10% Pd/C (100 mg) in methanol (10 mL) was stirred at 25 °C under hydrogen gas (1 atm, 25 °C) for 12 h. The soln was filtered through Celite and concd to yield methyl 3-*O*-(2-*O*-acetyl-3-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside (40 mg, 47% yield) which was used without further purification. Saturated methanolic ammonia (15 mL) was added, and the solution stirred at 25 °C for 3 h. Solvent was removed in vacuo, and the crude material was purified by silica gel chromatography (3:3:1 ethyl acetate:isopropanol:water) to yield **14** as a colorless glass (36 mg, 100% yield): ^{1}H NMR (D_2O): δ 3.30 (s, 3H); 3.35 (s, 3H); 3.50–4.03 (m, 12H); 4.66 (s, 1H); 5.09 (s, 1H); ^{13}C NMR (D_2O): δ 53.93; 53.9; 60.0; 60.1; 64.9; 64.9; 65.4; 68.7; 71 9; 72.5; 77.4; 78.8; 100.0; 101.5 ppm.; HRMS calc'd for $C_{14}H_{26}O_{11}$ 340.1475; found 370.1455.



Methyl 2,3,4-tri-*O*-benzyl-6-O-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside. *N*-Iodosuccinamide (47 mg, 0.2 mmol) was added to a soln of **25** (34 mg, 0.1 mmol) and **20** (93 mg, 0.2 mmol) in methylene chloride (10 mL), and the resulting soln was stirred with in the dark at 25 °C for 20 min. The reaction mixture was cooled to 0 °C and trifluoromethanesulfonic acid (5 mL of a 30 mM soln in methylene chloride) added. The reaction mixture was stirred at 0 °C for 2 h then filtered through Celite. The organic phase was washed with satd aq $NaHCO_3$ (2 × 20 mL), brine (2 × 20 mL) and 30% aq sodium thiosulfate solution (1 × 20 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 2,3,4-tri-*O*-benzyl-6-O-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside as an orange oil (70 mg, 100% yield): ^{1}H NMR ($CDCl_3$): δ 2.10 (s, 3H); 3.30 (s, 3H); 3.41 (s, 3H); 3.60–4.70 (m, 21H); 7.25–7.65 (m, 20H).

Methyl 6-*O*-(3-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside (15). To a soln of methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside (70 mg, 0.1 mmol) in absolute methanol was added 10% Pd/C (70 mg). The suspension was stirred under hydrogen gas (1 atm) at 25 °C for 8 h, filtered through Celite and concd. Methanol (20 mL) and solid potassium carbonate (50 mg, 0.4 mmol) were added The suspension was stirred at 25 °C for 8 h, filtered and concd. The crude product was purified by silica chroma-

tography (3:3:1 ethyl acetate:isopropanol: water) to yield **15** as an amorphous white solid (12 mg, 34% yield): ^{1}H NMR (D_2O): δ 3.37 (s, 3H); 3.40 (s, 3H); 3.56–3.78 (m, 5H); 3.82–3.99 (m, 6H); 4.20 (m, 1H); 4.75 (s, 1H); 4.91 (s, 1H); ^{13}C NMR (D_2O): δ 54.0 (s, OCH_3); 55.4 (s, 3-O-CH_3); 60.1 (s, 6a or 6b); 60.2 (s, 6a or 6b); 64.9 (s, 4a and 4b); 65.8 (s, 2a or 2b); 69.1 (s, 2a or 2b); 69.9 (s, 3a, 3b, or 5a); 70.0 (s, 3a, 3b, or 5a); 71.9 (s, 3a, 3b, or 5a); 79.1 (s, 5b); 98.7 (s, 1b); 100.3 (s, 1a); HRMS calcd for $C_{14}H_{26}O_{11}$ 370.1475; found 370.1538.



Methyl 6-*O*-*t*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside. *t*-Butyldiphenylchlorosilane (552 mg, 2.0 mmol) was added dropwise with stirring to a soln of methyl 1-thio-α-D-mannopyranoside (422 mg, 2.0 mmol) and imidazole (198 mg, 2.9 mmol) in dry tetrahydrofuran (15 mL). The soln was stirred at 25 °C for 8 h then extracted with methylene chloride (2 × 25 mL). The organic extract was dried ($MgSO_4$), concd and the residue purified by silica chromatography (ethyl acetate) to yield methyl 6-*O*-*t*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside as a white foam (400 mg, 44% yield): ^{1}H NMR ($CDCl_3$): δ 1.05 (3, 9H); 2.15 (s, 3H); 3.80–4.15 (m, 6H); 5.16 (s, 1H); 7.35–7.71 (m, 10H).

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside. A soln of methyl 6-*O*-*t*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside (400 mg, 0.9 mmol) and tetrabutylammonium bromide (86 mg, 0.3 mmol) in dry tetrahydrofuran (20 mL) was added dropwise with stirring at 0 °C to sodium hydride (118 mg, 3 mmol). The reaction mixture stirred at 0 °C under argon for 10 min, then benzyl bromide (504 mg, 3.0 mmol) was added. The soln was warmed to 25 °C and stirred for 8 h. The reaction mixture was quenched with methanol (3 mL) and extracted with chloroform (2 × 50 mL). The organic phase was washed with 1 M HCl (1 × 25 mL) and brine (2 × 50 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica chromatography (6:1 petroleum ether:ethyl acetate) to yield methyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside as a pale yellow oil (600 mg, 94% yield): ^{1}H NMR ($CDCl_3$): δ 1.08 (s, 9H); 2.09 (s, 3H); 3.88–4.30 (m, 6H); 4.60–5.07 (m, 6H); 5.36 (s, 1H); 7.37–7.89 (m, 25H).

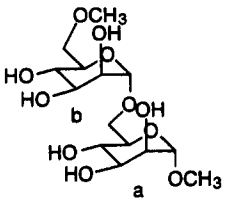
Methyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside. HF/pyridine (5 mL) was added dropwise with stirring to a soln of methyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside (600 mg, 0.8 mmol) in dry tetrahydrofuran (15 mL), and the soln was stirred at 25 °C for 8 h. The reaction mixture was extracted with methylene chloride (2 × 50 mL), washed with brine (2 × 50 m), dried ($MgCl_2$), and concd. The crude material was purified by silica chromatography (ethyl acetate) to yield methyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside as a clear oil (90 mg, 22% yield): ^{1}H NMR ($CDCl_3$): δ 2.08 (s, 3H); 3.75–4.03 (m, 6H); 4.55–4.72 (m, 6H); 5.20 (s, 1H); 7.25–7.55 (m, 15H).

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-1-thio-α-D-mannopyranoside (26). A soln of methyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (90 mg, 0.2 mmol) in dry tetrahydrofuran (15 mL) was added with stirring to sodium hydride (7.5 mg, 0.2 mmol). Iodomethane (26.7 mg, 0.2 mmol) was added, and the soln was stirred at 25 °C for 8 h. The reaction mixture was quenched with methanol (1 mL) and extracted with chloroform (2 × 25 mL). The organic phase was washed with brine (2 × 25 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-1-thio-α-D-mannopyranoside as a clear oil (30 mg, 32% yield): ^{1}H NMR ($CDCl_3$): δ 2.10 (s, 3H); 3.36 (s, 3H); 3.55–4.16 (m, 6H); 4.50–4.78 (m, 6H); 7.26–7,43 (m, 15H).

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside. A soln of methyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-1-thio-α-D-mannopyranoside (30 mg, 0.06 mmol), methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (56 mg, 0.12 mmol) in methylene chloride (10 mL) and *N*-iodosuccinamide (27.3 mg, 0.121 mmol) was stirred at 25 °C for 20 min. The reaction mixture was cooled to 0 °C and trifluoromethanesulfonic acid (5 mL of a 30 mM solution in methylene chloride) was added. The reaction mixture stirred at 0 °C for 2 h then filtered through Celite. The filtrate was washed with satd aq. $NaHCO_3$ (1 × 50 mL), brine (1 × 50 mL), and 10% aq sodium thiosulfate (1 × 50 mL), dried ($MgSO_4$), and concd. The crude material was purified by silica chromatography (4:1 petroleum ether:ethyl acetate) to yield methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside as a colorless oil (30 mg, 54% yield): ^{1}H NMR ($CDCl_3$): δ 3.20 (s, 3H); 3.30 (s, 3H); 3.43–4.07 (m, 11H); 4.40–5.02 (m, 15H); 7.19–7.40 (m, 30H).

Methyl 6-*O*-(6-*O*-methyl-α-D-mannopyranosyl)-α-D-mannopyranoside (16). A suspension of methyl 2,3,4-tri-*O* -benzyl- 6 -*O*- (2, 3, 4-tri -*O*- benzyl- 6 -*O*- methyl-α-D-mannopyranosyl)-ασ-D-mannopyranoside (30 mg, 0.03 mmol) and 10% Pd/C (30 mg) in methanol (7 mL) was stirred at 25 °C under hydrogen gas for 8 h. The reaction mixture was filtered through Celite and concd. The crude material was purified by silica chromatography (4:1 ethyl acetate:methanol) to yield **16** as a colorless glass (5.6 mg, 46% yield): ^{1}H NMR ($CDCl_3$): δ 3.35 (s, 3H); 3.37 (s, 3H); 3.42 (s, 1H); 3.50 (s, 1H); 3.52–3.62 (m, 3H); 3.64–3.88 (m, 5H); 4.09 (m, 1H); 4.38 (m, 1H); 4.75 (s, 1H); 4.88 (s, 1H); ^{13}C NMR (D_2O): δ 54.0 (OCH_3); 56.7 (6-O-CH_3); 60.1 (6a or 6b); 60.2 (6a or 6b); 64.9 (4a and 4b); 65.8 (2a or 2b); 69.1

(2a or 2b); 69.9 (3a, 3b, or 5a); 70.0 (3a, 3b, or 5a); 71.9 (3a, 3b, or 5a); 79.1 (5b); 98.7 (1b); 100.3 (1a); HRMS calcd for $C_{14}H_{26}O_{11}$ 370.1475; found 370.1473.



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References

1. Bremer, E. G. *Curr. Top. Membr. Res.* **1994**, *40*, 387.

2. Drickamer, K.; Taylor, M. E. *Annu. Rev. Cell Biol.* **1993**, *9*, 237.

3. Jungalwala, F. B. *Neurochem. Res.* **1994**, *19*, 945.

4. Lis, H.; Sharon, N. *Annu. Rev. Biochem* **1986**, *55*, 35.

5. Rademacher, T. W.; Parekh, R. B.; Dwek, R. A. *Annu. Rev. Biochem.*

6. Springer, T. A. *Annu. Rev. Cell Biol.* **1990**, *6*, 359.

7. Ingram, G. A.; Molyneux, D. H. In *Lectin Reviews*; Kilpatrick, D. C.; Van Driessche, E., Bøg-Hansen, T. C., Eds.; Sigma: St. Louis, 1991; Vol. 1, p 103.

8. Anderson, O., Lauesen, S. B.; Svehag, S.-E.; Holmskov, U.; Theil, S. In *Lectin Reviews*; Kilpatrick, D. C.; Van Driessche, E., Bøg-Hansen, T. C., Eds.; Sigma: St. Louis, 1991; Vol. 1, p 41.

9. Manocha, M. S.; Chen, Y. *Can. J. Microbiol.* **1990**, *36*, 69.

10. Gabius, H.-J.; Gabius S. In *Lectin Reviews*; Kilpatrick, D. C.; Van Driessche, E., Bøg-Hansen, T. C., Eds.; Sigma: St. Louis, 1991; Vol. 1, p 91.

11. Hiraizumi, S.; Takasaki, S.; Ohuchi, N.; Harada, Y.; Nose, M.; Mori, S.; Kobata, A. *J. Cancer Res.* **1992**, *83*, 1063.

12. Muramatsu, T. *Glycobiology* **1993**, *3*, 298.

13. Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321.

14. Mandal, D. K.; Kishore, N.; Brewer, C. F. *Biochemistry* **1994**, *33*, 1149.

15. Chervenak, M. C.; Toone, E. J. *Biochemistry* **1995**, *34*, 5685.

16. Schwarz, F. P.; Puri, K. D.; Bhat, R. G.; Surolia, A. J. *J. Biol. Chem.* **1993**, *268*, 7668.

17. Ramkumar, R.; Surolia, A.; Podder, S. K. *Biochem. J.* **1995**, *308*, 237.

18. Isbister, B. D.; St. Hilaire, P. M.; Toone, E. J. *J. Am. Chem. Soc.* **1995**, *117*, 12877.

19. Chervenak, M. C.; Toone, E. J. *J. Am. Chem. Soc.* **1994**, *116*, 10533.

20. Connelly, P. R.; Thomson, J. A.; Fitzgibbon, M. J.; Bruzzese, F. J. *Biochemistry* **1993**, *32*, 5583.

21. Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8069.

22. Murphy, K. P.; Privalov, P. L.; Gill, S. J. *Science* **1990**, *247*, 559.

23. Lee, B. *Meth. Enzymol.* **1995**, *259*, 555.

24. Sumner, J. B. *J. Biol. Chem.* **1919**, *37*, 137.

25. Leiner, I. E. In *Concanavalin A as a Tool*; Bittinger, H.; Schnebli, H. P., Eds.; Wiley: New York, 1976; p 17.

26. Goldstein, I. J.; Portez, R. D., in *The Lectins: Properties, Functions and Applications in Biology and Medicine*; Leiner, I. E., Sharon, N.; Goldstein, I. J., Eds.; Academic: New York, 1985; p 35.

27. Brewer, C. DF.; Bhattacharyya, L., Brown, R. D.; Koenig, S. H. *Biochem. Biophys. Res. Commun.* **1985**, *127*, 1066.

28. Derewenda, Z.; Yariv, J.; Helliwell, J. R.; Kalb, A. J.; Dodson, E.; Paiz, M., Wan, T., Campbell, J. W. *EMBO J.* **1989**, *8*, 2189.

29. Naismith, J. H.; Field, R. A. *J. Biol. Chem.* **1996**, *271*, 972.

30. Moreira, R. A.; Barros, A. C. H.; Stewart, J. C.; Pusztai, A. *Planta* **1983**, *158*, 63.

31. Nashed, M. A. *Carbohydr. Res.* **1978**, *60*, 200.

32. Leontein, K.; Nilsson, M.; Norberg, T. *Carbohydr. Res.* **1985**, *144*, 231.

33. Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L.-N. *Anal. Biochem.* **1989**, *179*, 131.

34. Grime, J. K. *Analytical Solution Calorimetry*; Wiley: New York, 1985.

35. Becker, J. W.; Reeke, G. N.; Edelman, G. M. *J. Biol. Chem.* **1971**, *246*, 6123.

36. Toone, E. J. *Curr. Opin. Structural Biol.*, **1994**, *4*, 719.

37. Weatherman, R. V.; Mortell, K. H.; Chervenak, M.; Kiessling, L. L.; Toone, E. J. *Biochemistry* **1996**, in press.

38. Buchanan, J. G.; Schwarz, J. C. P. *J. Chem. Soc.* **1962**, 4770; **1988**, *57*, 785.

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